

CLAIMS:

1. A method of screening for a substance or gene (termed herein "first gene") that affects activity or effect of a second gene, or activity or effect of a treatment, or activity or effect of a physiological function or behaviour, on a fish, the method comprising:

providing, as model fish for screening, (i) fish transgenic for the second gene, wherein the second gene is under regulatory control of a specific promoter and expression of the second gene within the fish affects an aspect of behaviour or physiology of the fish, or (ii) fish subject to said treatment, wherein the treatment affects an aspect of behaviour or physiology of the fish;

15 mutating said model fish to provide mutated fish or treating said model fish with a test substance to provide treated fish;

comparing an aspect of behaviour or physiology of mutated fish or treated fish with that aspect of behaviour or 20 physiology of model fish in order to identify any mutated fish or treated fish with altered behaviour or physiology compared with model fish;

thereby to identify a test substance that affects activity or effect of the second gene or activity or effect of 25 said treatment, or, by identifying a genetic difference between model fish and mutated fish with such altered behaviour or physiology to identify a first gene that affects activity or effect of the second gene or activity or effect of said treatment;

30 wherein the mutated fish or treated fish, and model fish, are subject to two opposing stimuli, and the aspect of behaviour or physiology of the mutated fish or treated fish subject to the two opposing stimuli is compared with the

aspect of behaviour or physiology of model fish subject to the two opposing stimuli.

2. A method according to claim 1 wherein either or both of
5 the two opposing stimuli are selected from any combination of
the members of the group consisting of: a visual stimulus,
light stimulation, optomotor stimuli, an audible startle
stimulus, discrete changes in temperature, a temperature
gradient, food, one or more aversive substances, one or more
10 attractive or addictive substances, physical aversion such as
electric shock, and a threatening shape.

3. A method according to claim 1 or claim 2 wherein fish are
habituated to an addictive substance, and one of the two
15 opposing stimuli is provided by a source of the addictive
substance accessible to the fish.

4. A method according to claim 3 wherein the addictive
substance is nicotine.

20 5. A method according to any one of claims 1 to 4 wherein
one of the two opposing stimuli is provided by a temperature
gradient in water inhabited by the fish.

25 6. A method according to claim 5 wherein the temperature
gradient is from 24°C to 40°C

7. A method according to any one of claims 1 to 6 wherein
one of the two opposing stimuli is provided by an inducer of
30 the optomotor response.

8. A method according to claim 7 wherein the inducer is
moving stripes.

9. A method according to any one of claims 1 to 8 comprising treating fish with two or more substances, at least one of which is a test substance, and comparing the effect of the two or more substances in combination, whether simultaneously or sequentially applied, on the aspect of behaviour or physiology of treated fish with the effect of one or more of the two or more substances when applied individually or alone.

10 10. A method according to claim 9 wherein treated fish are transgenic and/or mutated.

11. A method according to any one of claims 1 to 10 comprising providing fish transgenic for the second gene, wherein the second gene is under regulatory control of an inducible and/or tissue-specific promoter.

12. A method according to claim 11 wherein the promoter directs eye-specific expression.

20 13. A method according to claim 11 wherein the promoter directs ear-specific expression.

14. A method according to any one of claims 1 to 13 comprising determining auditory function of the fish.

25 15. A method according to any one of claims 1 to 14 comprising determining visual function of the fish.

30 16. A method according to any one of claims 1 to 15 comprising determination of pigmentation of the fish.

17. A method according to any one of claims 1 to 16 further comprising formulating a substance that affects activity or

effect of said second gene, or activity or effect of said treatment, into a composition comprising at least one additional component.

5 18. A method according to any one of claims 1 to 17 comprising identifying said first gene.

10 19. A method according to claim 18 further comprising screening for a test compound that affects activity of the first gene.

20. A method according to claim 18 further comprising screening for a test compound that interacts with a protein encoded by the first gene.

15 21. A method according to claim 19 or 20 further comprising formulating a test compound that affects activity of the first gene or that interacts with a protein encoded by the first gene into a composition comprising at least one additional component.

25 22. A method according to any one of claims 1 to 21 comprising treating fish with a substance dissolved in a solvent, wherein the solvent is dimethyl sulphoxide (DMSO), methanol or ethanol.

23. A method for determining effect of a substance on a fish, the method comprising applying the substance dissolved in dimethyl sulphoxide (DMSO) to water inhabited by the fish.

30 24. A method according to claim 22 or claim 23 wherein the substance dissolved in DMSO is added to water inhabited by the fish to give a final concentration of DMSO of 1% or less.

25. A method of screening for an optimum combination of two substances, drugs, genes or drug targets that affect activity or effect of a second gene, or activity or effect of a treatment, or activity or effect of a physiological function or behaviour, on a fish, the method comprising:

providing, as model fish for screening, (i) fish transgenic for the second gene, wherein the second gene is under regulatory control of a specific promoter and expression of the second gene within the fish affects an aspect of behaviour or physiology of the fish, or (ii) fish subject to said treatment, wherein the treatment affects an aspect of behaviour or physiology of the fish;

mutating said model fish to provide mutated fish with two mutations and/or treating said model fish with a test substance or two test substances to provide treated fish;

comparing an aspect of behaviour or physiology of mutated fish or treated fish with that aspect of behaviour or physiology of model fish in order to identify any mutated fish or treated fish with altered behaviour or physiology compared with model fish;

thereby to identify a combination of two substances, drugs, genes or drug targets that affects activity or effect of the second gene or activity or effect of said treatment, or, by identifying a genetic difference between model fish and mutated fish with such altered behaviour or physiology to identify a first gene and/or further gene that affects activity or effect of the second gene or activity or effect of said treatment.

30 26. A method according to claim 25 wherein a first substance or drug is administered at an equal concentration in multiple wells, and to each individual well a further substance or drug is added, and the effect of each substance or drug in various combinations is compared to determine the optimum combination.

27. A method according to claim 26 wherein the effect of each further substance or drug in various combinations is compared to determine deleterious combinations.

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28. A method according to claim 25 comprising determining the optimum combination of two potential drug targets, by comparing the effect of mutations or genetic alterations in various combinations.

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29. A method according to any one of claims 25 to 28 comprising testing combinations of 3, 4, 5 or any higher number of substances, drugs, genes or drug targets.

15 30. A method of determining whether two or more substances, drugs, genes or drug targets that affect activity or effect of a second gene, or activity or effect of a treatment, or activity or effect of a physiological function or behaviour, on a fish, have an additive or synergistic effect when present 20 simultaneously, the method comprising:

providing, as model fish for screening, (i) fish transgenic for the second gene, wherein the second gene is under regulatory control of a specific promoter and expression of the second gene within the fish affects an aspect of behaviour or physiology of the fish, or (ii) fish subject to said treatment, wherein the treatment affects an aspect of behaviour or physiology of the fish;

25 mutating said model fish to provide mutated fish with one or two mutations and/or treating said model fish with a test 30 substance or two test substances to provide treated fish;

comparing an aspect of behaviour or physiology of mutated fish or treated fish with that aspect of behaviour or physiology of model fish in order to identify any mutated fish or treated fish with altered behaviour or physiology compared

with model fish; wherein comparison is made between application of mutations and/or test substances individually and in combination;

thereby to determine whether a combination of one or two substances, drugs, genes or drug targets that affects activity or effect of the second gene or activity or effect of said treatment, and/or one or two genetic differences between model fish and mutated fish with such altered behaviour or physiology has an additive or synergistic effect.

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31. A method according to claim 30 comprising administering two substances or drugs separately and in combination.

32. A method according to claim 31 wherein the effects of two substances or drugs both separately and in combination are assayed at a range of possible combinations from their respective dose-response curves when given in isolation.

33. A method according to claim 31 or claim 32 wherein the effect when given in combination is compared to see whether it is greater than the summation of the effect of the two substances or drugs at the same concentration given separately.

34. A method according to claim 30 comprising determining the additive or synergistic effect of two or more potential drug targets, by comparing the effect of mutations or genetic alterations in various combinations.

35. A method according to any one of claims 30 to 34 comprising testing combinations of 3, 4, 5 or any higher number of substances, drugs, genes or drug targets.

36. A method of identifying a substance, drug, gene or drug target that lessens an otherwise deleterious effect or side effect of a second substance, drug, gene or drug target, the method comprising:

5 providing, as model fish for screening, (i) fish transgenic for the second gene, wherein the second gene is under regulatory control of a specific promoter and expression of the second gene within the fish affects an aspect of behaviour or physiology of the fish; or (ii) fish subject to
10 said treatment with said second substance, wherein the treatment affects an aspect of behaviour or physiology of the fish;

15 mutating said model fish to provide mutated fish with one or more mutations and/or treating said model fish with one or more two test substances to provide treated fish;

20 comparing an aspect of behaviour or physiology of mutated fish or treated fish with that aspect of behaviour or physiology of model fish in order to identify any mutated fish or treated fish with altered behaviour or physiology compared with model fish; wherein comparison is optionally made between application of mutations and/or test substances individually and in combination;

25 thereby to determine whether a substance, drug, gene or drug target affects activity or effect of the second substance, drug, gene or drug target, whereby a substance that lessens said otherwise deleterious effect or side effect is identified.

30 37. A method according to claim 36 wherein said second substance, drug, gene or drug target has a beneficial effect, but also a negative effect in isolation, whereas in combination with the test compound, the negative effect is lessened.

38. A method according to claim 36 or claim 37 wherein the effect is assayed in multiwell format with a range of possible ameliorating compounds or genetic mutations.

5 39. A method according to any one of the preceding claims wherein a gene or mutation is identified for a patient population which is more likely to respond to a particular substance or drug, or which is less likely to respond to a particular substance or drug, or which may demonstrate a
10 negative side effect when administered a particular substance or drug.

40. A method according to claim 39 wherein the effect of a particular mutation or polymorphism on the efficacy or side
15 effect profile of a test compound or genetic alteration is compared with wild type response.

41. A method according to claim 39 or claim 40 wherein the effect is assayed in a medium or high throughput fashion in
20 zebrafish to identify such possible polymorphisms or genetic factors resulting in variations in drug responsiveness.

42. A method according to any one of claims 39 to 40 wherein one or more genetic factors identified in zebrafish are used
25 to identify corresponding human genetic factors.